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Application No. 09/581,861  
Amendment And Response  
Reply to Office Action of August 24, 2006

Docket No.: 60623(50370)

**AMENDMENTS TO THE CLAIMS**

Please amend claims 1, 53, 59, and 121 and please add claims 123-131. The below listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and

a chimeric G protein subunit which comprises

an endogenous yeast Gpa1 subunit having C and N terminal amino acids in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different;

such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

2-52. (Cancelled)

53. (Currently amended) A recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR

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acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit having C and N terminal amino acids in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which at least the first five N-terminal amino acids of said Gpa1 are replaced with at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different; such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

54. (Original) The yeast cell of claim 53, wherein said chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

55-56. (Cancelled)

57. (Previously presented) The yeast cell of claim 1, wherein in said chimeric G protein subunit, the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a heterologous G protein subunit.

58. (Cancelled)

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59. (Currently amended) A chimeric G-protein subunit which comprises an endogenous Gpa1 subunit having C and N terminal amino acids in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different.

60. (Original) The chimeric G-protein subunit of claim 59, in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of said second heterologous G protein subunit.

61-119 (Cancelled).

120. (Previously presented) The yeast cell of claim 1 or 53, wherein an endogenous yeast pheromone receptor protein is not produced in functional form.

121. (Currently amended) The yeast cell of claim 1 or 53, further comprising an indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein-coupled receptor to the G protein.

122. (Previously presented) The yeast cell of claim 1 or 53, wherein the yeast cell is a *Saccharomyces cerevisiae* cell.

123. (New) The yeast cell of claim 1, wherein the heterologous G protein-coupled receptor (GPCR) is selected from the group consisting of FPR1, GalR1, VIPR, ML1aR, ML1bR, C5aR, FPRL1, IL-8R, A2aR, NocR, SSTR2, NTR and MC4R.

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124. (New) An assay to identify compounds capable of modulating the dissociation of G $\alpha$  and G $\beta\gamma$ , comprising the steps of:

- (i) providing a yeast cell according to claim 1 or 53,
- (ii) contacting the yeast with a test compound; and
- (iii) identifying compounds which induce a change in a detectable signal in the yeast cell, wherein said change in a detectable signal indicates dissociation of G $\alpha$  and G $\beta\gamma$ .

125. (New) The assay of claim 27, wherein said test compound is from a library of non-peptidic organic molecules.

126. (New) A method for identifying a compound that modulates a heterologous G protein-coupled receptor, comprising the steps of:

- (i) providing a yeast cell according to claim 1 or 53,
  - (ii) contacting the yeast with a test compound; and
  - (iii) determining whether the test compound induces a change in a detectable signal in the yeast cell, wherein a change in detectable signal indicates that the test compound modulates the receptor;
- to thereby identify a compound that modulates a heterologous G protein-coupled receptor.

127. (New) The assay of claim 126, wherein said test compound is from a library of non-peptidic organic molecules.

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128. (New) The assay of claim 126, wherein said yeast cell further comprises an indicator gene that produces said detectable signal.

129. (New) The assay of claim 128, wherein said indicator gene is selected from the group consisting of HIS 3,  $\beta$ -galactosidase, alkaline phosphatase, horseradish peroxidase, exoglucanase, luciferase, BAR1, PHO5, green fluorescent protein and chloramphenicol acetyl transferase.

130. (New) The assay of claim 129, wherein said indicator gene is selected from the group consisting of HIS 3,  $\beta$ -galactosidase and green fluorescent protein.

131. (New) The assay of claim 126, wherein the heterologous G protein-coupled receptor is an orphan receptor.